

## Dwarf pea response to gibberellic acid applied to soil through a drip irrigation system, and gibberellic acid biodegradation in soil

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### Abstract

Gibberellic acid (29 or 290  $\mu\text{M}$ ) injected into drip irrigation lines significantly stimulated internode elongation of dwarf peas, and the 290- $\mu\text{M}$  soil treatment produced significantly taller plants than did the 29- $\mu\text{M}$  treatment.  $\text{GA}_3$  uptake may limit  $\text{GA}$ -induced internode elongation when  $\text{GA}_3$  is applied to soil, in contrast to results obtained for hydroponically grown plants, where uptake initially appeared to exceed the rate of hormone metabolism (Anderson *et al.*). It is likely that biodegradation or chemical inactivation limited the plant-availability of  $\text{GA}_3$  in the soil. Degradation of moderate  $\text{GA}_3$  concentrations in a moist, aerobic loamy fine sand was nearly complete within five days, indicating that the inefficiency of soil applications may outweigh the benefits provided by reducing labor costs associated with foliar-spray applications.

### Introduction

Gibberellins (GAs) typically are applied to commercial crops by foliar spraying, but in some instances it may be preferable to add the hormone to the soil, although plant response to soil-applied  $\text{GA}$  will depend upon both the rate of  $\text{GA}$  biodegradation in the soil and the extent of  $\text{GA}$  adsorption onto soil solids. Soil applications of GAs have been shown to promote conifer growth as much as does foliar spraying (Little and Loach, 1975), but other research has indicated that response to gibberellic acid ( $\text{GA}_3$ ) in the soil is less than in sand (Katznelson and Cole, 1965), sterile soil (Brian *et al.*, 1954), or solution culture (Arteca *et al.*, 1985). However, the growth data in the above studies were not normalized relative to the growth of control plants grown in each medium, and such normalization might affect the interpretation of the results.

To date the only published reports of  $\text{GA}_3$  biodegradation in soils have been qualitative and inferential, based upon plant response to  $\text{GA}_3$  in the soil (Brian *et al.*, 1954; Katznelson and Cole, 1965).

Therefore, the objectives of this research were to determine the rate of  $\text{GA}_3$  loss from a soil and to compare the response, defined as normalized elongation rates, of  $\text{GA}_3$ -treated, hydroponically grown dwarf pea plants (Anderson *et al.*, 1988) to the response of pea seedlings to soil applications of  $\text{GA}_3$ .

### Methods

#### Soils

The soil material used in the glasshouse and laboratory experiments was a coarse loamy, mixed, non-acid, thermic Typic Xerorthent (Hanford loamy fine sand from Riverside, California) which was passed through a 2-mm sieve without further drying; the field-moist soil contained less than 2% water (oven-dry basis). Sieved soil was used in glasshouse and laboratory incubation experiments (Table 1).

Table 1. Properties of Hanford loamy fine sand (Typic Xerorthents)

pH	Organic C g kg <sup>-1</sup>	Saturation %	Initial EC <sub>e</sub> dS m <sup>-1</sup>	Final EC <sub>e</sub> dS m <sup>-1</sup>
6.64 ± 0.03 <sup>a</sup>	0.15 ± 0.02	30.6 ± 0.9	1.02 ± 0.08	0.87 ± 0.10

<sup>a</sup> Mean of three replicates ± sample standard deviation

### Glasshouse

Dwarf peas (*Pisum sativum* L., cv. Little Marvel) were sown directly into 7.6-l pots containing 7 kg of soil. After emergence the seedlings were thinned to one plant per pot. A drip emitter was placed at the base of each plant, and a pvc ring 12.7 cm in diameter was pressed into the soil around each seedling to prevent irrigation water from channeling along the pot-soil interface. All treatments received equal volumes of water in daily irrigation cycles regulated by an automatic timer. Water applications were adjusted during the experiment to compensate for higher water consumption as the plants grew larger. Gibberellic acid (Sigma Chemical) was injected into the irrigation system with Dosamatic liquid dispensers (J. F. Equipment Company, Dallas, TX) eighteen days after planting. Three different GA<sub>3</sub> treatments (0, 29, or 290 μM GA<sub>3</sub>) were used. The treatments were assigned in a randomized block design, with 8 replicates per treatment. Treatments (135 ml of GA<sub>3</sub> solution or deionized water for the control) were applied six times daily at two-hour intervals over a two-day period. The total solution volume added was calculated to bring the soil to  $0.7 \times \theta_{\text{sat}}$ , with total GA<sub>3</sub> doses of 0, 2.2, or 22 mg kg soil<sup>-1</sup> (0, 44.5, 445 μmol per plant). The pots had holes at the bottom to allow drainage, but leaching of GA<sub>3</sub> from the pots was presumed to be negligible because the initial hormone-containing irrigations did not drain from the pots and no additional irrigations were required during the week immediately following treatment.

Internode lengths were determined 0, 4, 8, 12, 18, and 24 days after treatment (DAT). Elongation rates and the elongation response were calculated as described in a previous paper (Anderson *et al.*, 1988). In addition, elongation rates were normalized so that growth of soil-grown plants could

be compared with that of hydroponically grown plants, using the equation

$$\text{Normalized elongation rate (NER)} = \frac{(\text{Elongation rate of treated plants grown in either soil or nutrient solution})}{(\text{Mean elongation rate of untreated control plants grown in same medium})} \times 100$$

### Incubations

Two hundred and fifty g of unamended Hanford soil were incubated aerobically at 24°C in the dark with 50 mg kg<sup>-1</sup> GA<sub>3</sub> (approximately 2.3 times the application rate used in the glasshouse experiment) at a soil water content of  $0.6 \times \theta_{\text{sat}}$  for 0, 5, or 10 d in 1-l Erlenmeyer flasks, with three replicates per sampling date. At the end of the incubation period (1 h for the 0-day incubation) GA<sub>3</sub> was extracted from the soils with 0.01 M KH<sub>2</sub>PO<sub>4</sub>, pH 7.4, in two sequential extractions using a 2:1 solution:soil ratio in each extraction. The extract was concentrated under vacuum at 40°C and analyzed by reverse-phase HPLC using a methanol/water solution containing 0.01 M H<sub>3</sub>PO<sub>4</sub> as the eluent. The solvent gradient was from 20 to 30% methanol in 6 minutes, with a flow rate of 1.8 ml min<sup>-1</sup> (Anderson and Jarrell, 1988).

## Results

### Incubation

The samples extracted at  $t = 0$  days (d) were incubated for 1 h prior to extraction, and approximately 10% of the added GA<sub>3</sub> was not recovered after this brief incubation with the soil (Fig. 1). If the kinetics of adsorption are much more rapid than the rate of biodegradation, then most, if not all, of the initially non-extractable GA<sub>3</sub> must have been adsorbed by the soil. Similarly, if adsorption were nearly instantaneous and essentially time-independent, then the difference between the amount of GA<sub>3</sub> extracted from the soil at  $t = 0$  d and that extracted at later dates may be estimated by calculating the difference between the amount extracted at  $t = 0$  and the amount extracted after longer incubation.

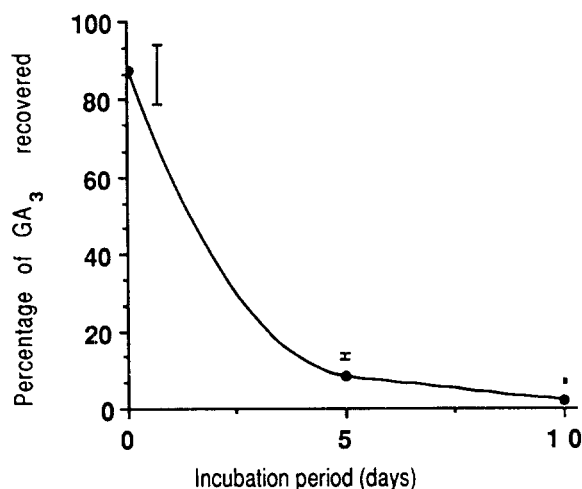


Fig. 1. Time course of GA<sub>3</sub> extractability from Hanford loamy fine sand. Each point represents the mean of three replicates, with error bars denoting the standard error of the mean.

The amount of extractable GA<sub>3</sub> decreased significantly during the first 5 d of the incubation (Fig. 1): approximately 85% of the GA<sub>3</sub> not initially adsorbed was degraded during the first five days. At the conclusion of the 10-d incubation period, the amount of GA<sub>3</sub> in the concentrated soil extracts was less than the HPLC detection limit (< 4.5% of the total amount of GA<sub>3</sub> originally added to the soil).

#### Drip-irrigation applications

Gibberellic acid additions to Hanford soil produced plants which always were significantly taller than untreated plants (Table 2). At all measurement dates the highest GA<sub>3</sub> concentration produced the tallest plants, although the difference was not significant ( $p > 0.05$ ) 4 DAT. The elongation response for plants treated with 29  $\mu\text{M}$  GA<sub>3</sub>

Table 2. Elongation response (percentage of control elongation) of pea seedlings following soil application of GA<sub>3</sub>

GA <sub>3</sub> conc., $\mu\text{M}$	Days after treatment				
	4	8	12	18	24
29	278	214	185	160	160
290	327	296	284	269	280
SE <sub>Δx</sub>	36	32	25	27	31
LSD <sub>0.05</sub>	76	68	52	58	66

Table 3. Elongation rates (cm/d) of pea seedlings following soil applications of GA<sub>3</sub>

GA <sub>3</sub> conc. $\mu\text{M}$	Days after treatment				
	4	8	12	18	24
0	1.01	1.46	1.26	0.54	0.00
29	2.77	2.58	1.58	0.38	0.00
290	3.47	4.23	3.20	1.14	0.38
SE <sub>Δx</sub>	0.21	0.44	0.30	0.34	0.13
LSD <sub>0.05</sub>	0.44	0.91	0.61	0.51	0.28

decreased steadily between 4 and 12 DAT, whereas the elongation response for those plants treated with 290  $\mu\text{M}$  GA<sub>3</sub> decreased very little during the same period (Table 2).

Gibberellic acid additions to the soil also enhanced elongation rates of treated plants. Treatment with 290  $\mu\text{M}$  GA<sub>3</sub> stimulated internode elongation rates at all times, while plants treated with 29  $\mu\text{M}$  GA<sub>3</sub> had higher rates of elongation than the control only during the initial week after treatment (Table 3). Although the 290- $\mu\text{M}$  treatment produced greater elongation rates than the 29- $\mu\text{M}$  treatment at all times, the increase was only significant 8 and 12 DAT, as well as 24 DAT when the mean elongation rate for control plants and those treated with 29  $\mu\text{M}$  GA<sub>3</sub> was zero.

#### Discussion

Gibberellic acid applied to Hanford loamy fine sand significantly stimulated internode elongation of dwarf pea seedlings, but the normalized elongation rate (NER) 8 DAT for the soil treatments was significantly less than for 3-d hydroponic treatment with equivalent concentrations (Fig. 2). Twelve DAT the response for 29  $\mu\text{M}$  GA<sub>3</sub> soil treatment was significantly less than that for hydroponically grown plants whose roots were in contact with an equivalent GA<sub>3</sub> concentration for 3 d ( $p \leq 0.05$ ; Fig. 2), but for plants treated with 290  $\mu\text{M}$  GA<sub>3</sub> there was no difference between the two media. By 18 DAT the NER depended only upon concentration; for a given GA<sub>3</sub> concentration there was not a significant difference between the media ( $p > 0.05$ ). the normalized elongation rate for soil-grown plants could not be calculated 24 DAT because the elongation rate for control plants was zero.

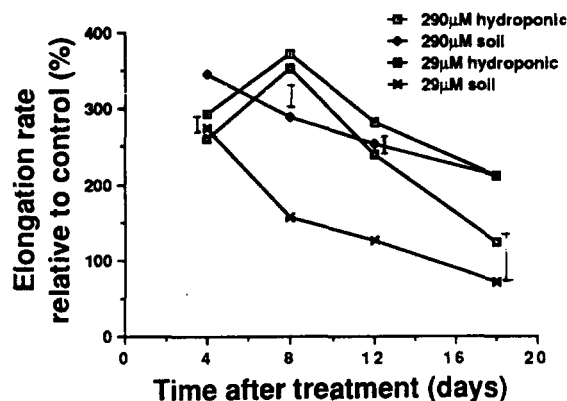


Fig. 2. Normalized elongation rates (percentage of control elongation rate) for soil applications or 3-d hydroponic treatment with  $GA_3$ . Error bars denote the standard error of the difference of means for soil-grown plants.

The NER of plants treated with  $29\mu M$   $GA_3$  usually depended upon growth medium, while the NER of the  $290\mu M$  soil treatment was not significantly different than that for 3-d hydroponic treatments at most sampling dates. This suggests that when  $GA_3$  is added to the soil, a fixed quantity, independent of the amount added, becomes unavailable to the plants within a few days. When a  $29\mu M$   $GA_3$  solution is applied to soil, the amount rendered unavailable apparently constitutes a major portion of the total, while for the  $290\mu M$  treatment the amount of the hormone available for plant uptake was large enough to produce a response comparable to that produced by  $GA_3$  in sterile nutrient solution for 3 d. In contrast to results obtained in hydroponic solution (Anderson *et al.*, 1988), application of  $29\mu M$   $GA_3$  to the soil was not a "saturating" hormone dose since the

$290\mu M$  treatment produced significantly taller plants than did the  $29\mu M$  treatment.

In addition, the steady decline of the normalized elongation rate for soil treatments suggests that  $GA_3$  uptake from soils limited the plant response, probably as a result of hormone degradation in the soil. This is in accordance with the results of the incubation and extraction study, which indicated that approximately 90% of added  $GA_3$  was degraded during a 5-d incubation. These results indicate that under these moisture and temperature conditions  $GA_3$  applications to medium-textured soils may be too inefficient to be economically feasible although other irrigation scheduling may favor plant uptake over degradation of the hormone. In addition,  $GA_3$  application through drip irrigation may lead to better plant response and greater cost-efficiency in coarser soils such as those often used for commercial grape production.

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